Experimental Studies of the Multitrophic Effects Of Anti-Herbivore Defense in Three Pine Barrens Shrub Species

by

Elizabeth R. Coffey

A Thesis

Submitted to the University at Albany, State University of New York

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

College of Arts & Sciences

Department of Biology

2012

Abstract

Plants serve as the foundation of two ecosystem trophic levels. Above ground, they are the primary food source for animals, while their roots and deposited leaves interact with the soil-dwelling community below ground. Plants have evolved the ability cope with herbivory and other environmental stressors by producing a number of chemical defense compounds that are manufactured through complex chemical reactions within the leaves and result in an array of direct and indirect defense responses, much like our own immune system. Induced chemical defenses potentially influence soil dynamics by causing changes in the nutrient ratio of the leaves, which are then seasonally shed and contribute to the organic layer of the soil, or by affecting the detritus community through leaching of chemicals in the root zone. Manual clipping was utilized in this study to induce the production of chemical compounds with the goal of examining the secondary effects of induced plant defenses. Soil invertebrate communities were assessed subsequent to the removal of 25% of the plant mass of three plant species in the Albany Pine Bush to detect downstream consequences of plant defense mechanisms. I also compared the leaf chemistry, whole plant growth and ensuing herbivory rates of damaged and undamaged *Salix humilis, Quercus prinoides,* and *Q. iliciofolia* shrub species. Thirty plants of each species were examined, half of which were treated by clipping annually for two consecutive years. Subsequent to final treatment, Nitrogen and Carbon contents of fallen leaf samples were analyzed periodically. A Berlese funnel extraction method was used to identify invertebrates from soil samples taken within 120 mm of the main plant stem for twenty plants from each species, ten of each treatment group. Soil pH was also determined for all soil samples taken for invertebrate analysis. Results indicate that soil invertebrate abundance and richness was significantly lower in the treatment groups of two of the three plant species. Soil samples from

untreated plants revealed 2.62 times more soil invertebrate abundance and included 25% more groups of taxa. Soil invertebrate populations were not correlated with soil pH or closest plant species. These findings support the claim that soil communities are influenced by the changes caused by induced plant defense mechanisms. Variation in soil invertebrate communities between treatment groups did not correspond to differences in plant chemistry, as C:N did not vary between damaged versus undamaged leaves. Possible explanations for this include the possibility that changes were not reflected in leaf litter samples due to the reabsorption of many nutrients prior to leaf shedding, a concentration of defense chemicals towards more sensitive parts such as seeds and roots, or that soil invertebrates are more influenced by induced chemical changes that occur in the root region. It was found that C:N varied significantly among plant species and sampling periods, and that the ratio of browsed branches one year after treatment was different among treatment groups and plant species. Treated dune willow shrubs had a reduced number of browsed branches, indicating a possible whole-plant response. This study displays the importance of preserving these unique simplistic system composed of an arid environment with poor, relatively homogenous soils and redundant vegetation, such as the Albany Pine Bush, which allow for an exceptional opportunity to investigate plant-soil interactions.

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AKNOWLEDGEMENTS

I extend my sincere gratitude to my advisor and committee chair, Dr. George Robinson, for his advice, guidance, and support during this study. His knowledge and expertise were crucial in completing this work. I would also like to thank my collaborator, Dr. Kaori Shiojiri, who was fundamental in the design and execution of this study. In addition, I thank my committee members, Mr. Neil Gifford and Dr. Andrei Lapenas, for their patience, assistance, and knowledge. My thanks also goes out to Dr. Gary Kleppel, BCP program director, for his continued support and guidance. I also am grateful for the support and assistance of my fellow graduate students, especially for the statistical expertise of Larissa Uusitalo and GIS input from Paul Sesink Clee and Matt Mitchell. I thank the SUNY Benevolant Association for financial support and the Biodiversity, Conservation and Policy program and Biology Department of the University at Albany for additional support and resources. Finally, I would like to express my abundant gratitude for the love, laughs, and support from friends and family throughout this endeavor.

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INTRODUCTION

A. PLANT DEFENSE

Trophic interactions are the fundamental properties of an ecosystem that dictate interspecific relationships by regulating the flow of nutrients. Plants contribute both living matter and detritous to terrestrial food webs. This includes detritivore communities at both the root region and at the soil surface, through root turnover and the seasonal contribution of leaves, the most dynamic component of soils. Plants are subject to herbivory both above and below ground, and have evolved complex chemical defense mechanisms in response. Induced chemical changes may cause alterations in the nutrient ratios within the plant, which are passed to the ecosystem and result in systematic changes in nutrient availability. This study took the unique approach of examining the relationship between levels of the food web by manipulating the chemical properties of primary producers through induction of chemical defense mechanisms, and examining the subsequent effects on the detritus food web. Understanding the complex consequences of the multitrophic interactions between plants and herbivores is a very active area of ecological research.

A.1. Evolution of Plant Defense

Herbivory has existed as a threat to plants throughout their evolutionary history, and has greatly influenced both plant populations and community structure. Generations can be lost through granivory, populations may be spatially limited due to herbivore activity, and general fitness may be decreased if the plant is attacked at certain anatomical locations or during crucial life stages (Hawkes and Sullivan, 2001). Herbivory is therefore generally considered harmful to plants, and can reduce growth and

reproductive ability (Gurevitch *et al.*, 2006). Plants are often subject to chronic or seasonally heavy herbivory, which places selective pressures on populations to evolve defense strategies. One response that has evolved frequently is the ability to change chemical and physical properties in an effort to deter herbivory. While some plants have developed ways to escape attack spatially, temporally, or morphologically, others have developed more specialized strategies through which to minimize the effects of herbivory on fitness and reproduction (Gurevitch *et al*., 2006). In particular, plants can muster a formidable array of defense chemicals, including toxins and feeding deterrents, in response to herbivory. Chemical defenses are sometimes generalized, but more often show a high degree of specificity that indicate co-evolution.

A.2. Plant Chemical Defense Compounds

Plant defense mechanisms can be broken into two broad categories. Constitutive defense mechanisms are always present within a plant and include thorns, trichomes, leaf toughness, or spines, as well as some chemical compounds. Induced defense mechanisms are produced by specific reaction mechanisms initiated by stress cues and signals. Induced chemical compounds include direct defenses that reduce palatability or digestability, act as toxins, or repell insects (Vet, 1999), as well as indirect defenses, which are synthesized compounds that attract natural predators of herbivores (Vet, 1999). Indirect defenses include provisionsal strategies, such as providing food and shelter to mutualist protectors, as well as reactionary measures, such as the release of volatile secretions to ward off attackers by attracting predators of that insect (Vet, 1999). Both indirect and direct defense strategies vary greatly among organisms and induced chemical blends may contain more than one hundred compounds (Dicke, 1999). Defense

compound combinations differ among plant species, genotype, and part, and in response to different herbivore species, stages, and densitites, as well as to abiotic factors (Vet, 1999). The types and quantity of defenses a plant are able to employ are often dictated by the resources available within the surrounding environment (Coley, *et al*., 1985). It appears that chronic herbivory selects more for constitutive defenses, while more sporadic herbivory selects for induced defenses because of the costs associated with producing defense compounds (Gurevitch *et al*., 2006). Chemical synthesis is governed by the cost of production, the level of efficacy, the risk of herbivory, and the cost of damage (Mooney, Gulman, and Johnson, 1983).

The synthesis of defense compounds has been repeatedly shown to increased fitness and survival. Agrawal (1998) showed that changes in leaf properties caused by herbivory increased seed production in plants by over 60%. In this case, induced responses decreased herbivory due to chewing herbivores and phloem-feeding aphids, indicating enhanced resistance to herbivory following attack (Agrawal, 1998). In some cases, such as in sagebrush (*Artemisia tridentata*), plants became more resistant to hervibory after exposure to volatile cues from experimentally damaged neighbors (Karban, *et al*., 2009). It was also found that resistance induced through clipping caused a release of defense volatiles that resulted in decreased herbivore damage in both the plants that were clipped (sagebrush) and neighboring reference tobacco plants (Karban, *et al*., 2000).

A textbook example of an evolved induced defense is displayed by the white clover, *Trifolium repens*, which releases cyanogenic glycosides in response to leaf damage (Dirzo and Harper, 1982). Defense compounds are activated and mobilized by

the presense of herbivorous snails to result in a higher survival rate in the presense of herbivory, but in the absense of snails, plants with the ability to produce cyanogenic glycosides had reduced growth and reproduction when compared with acyanogenic plants (Dirzo and Harper, 1982), indicating selective pressure for this costly adaptation to be displayed only in the presense of predatory snails. Dirzo and Harper (1982) supported this evolutionary theory by showing that areas with high snail densities had an increased percentage of cyanogenic individuals compared to those areas with smaller populations of herbivores. Much like our own immune system, these changes have evolved to increase the immediate robustness and fitness of an organism and to function as a deterrent to subsequent attack.

A.3. Induced Plant Defense Mechanisms

Induction begins with the production of volatiles, systematically released from both damaged and undamaged leaves, so that damage to only a few leaves results in a systemic response (Tumlinson, Pare and Lewis 1999). A 'wound messenger', systemin, is transmitted through the entire plant in response to damage, and promotes the synthesis and release of defense chemicals *de novo* (Tumlinson, Pare and Lewis1999). Hormones, such as jasmonates, communicate the occurence of an herbivore attack, and can promote a range of responses, from a highly specific reactions to certain invertebrate salivas, to a more generalized reaction due to mechanical damage (Gurevitch *et al*., 2006). The messenger causes a cascade of changes in the chemistry of the entire plant, often resulting in the utilization of primary or secondary metabolites to defend the plant against herbivory (Gurevitch *et al*., 2006).

Jasmonic acid is the jasmonite primarily responsible for induced defense and plant response to wounding (Hu, *et al*., 2009). This hormone is also related to growth, senescence and leaf abscission (Hu, *et al.,* 2009). Wound trauma causes the initiation of a signal transduction pathway that includes a system of calcium ion fluxes, membrane potentials, and phosphorylation cascades within 30 minutes of being damaged (Howe and Jander, 2008) and results in the induction of direct and indirect defense mechanisms. The specific mechanisms of response to stress signals, including wounding, have been detailed by numerous studies (Howe and Jander, 2008; Hu, *et al*., 2009; Delker, 2006). Secondary metabolites include three main types: phenolics, alkaloids, and terpenes (Gurevitch *et al*., 2006). Plants often use combinations of these substances in various forms to reduce subsequent attack, to induce defensive responses in neighboring plants, or to attract natural enemies of the herbivores (Pare and Tumlimson, 1999). Phenols include substances like tannins, which reduce digestability, lignin, which acts as a structural barrier, poisons such as saponins, and other compounds including flavonoids and anthocyanins (Gurevitch *et al*., 2006). Alkaloids are bitter, toxic compounds that are often highly specific and effective in small quantities, such as caffiene, nicotiene, and cocaine (Gurevitch *et al*., 2006). There are a great variety of terpenes, which can act as toxins, detterants, or poisons, and in some plants mimic insect molting hormones (phytoecdysones) and disrupt insect development (Gurevitch *et al.,* 2006).

Although numerous factors lead to a great deal of variability in the quantity, quality, and timing of induced defense, all are instigated through the jasmonic pathway (Wang, *et al*., 2000). The production of defense compounds therefore causes changes in

plant chemistry that may impact soil invertebrates through the root zone, or affect detritivores when leaves are seasonally shed.

B. Influence of Induced Plant Responses on Detritivore Communities

Decomposition of litter is a vital process in ecosystems that regulates nutrient cycles and plays a fundamental role in determining ecosystem productivity (Enokie and Kawaguchi, 2000). The rate of decomposition is determined by several factors, including litter quality, soil moisture, and detritivores (Enoki and Kawaguchi, 2000). Leaves of deciduous trees are broken down through the activities of fungi, invertebrates, and bacteria that transport, digest, convert, and recycle nutrients..

Nutrient ratios determine the rate of decomposition. Increased nitrogen and phosphorus concentrations were shown to increase leaf breakdown rates, microbial respiration rates, and macroinvertebrate biomass (Greenwood, *et al*., 2007). Simalarly, the biomass of macroinvertebrates in lower quality (higher C:N) soils was more responsive to nutrient enrichment (Greenwood, *et al*., 2007). As resource availability changes, the soil community responds because the nutritional and chemical makeup of soils often affects soil invertebrate populations. In the rhizosphere their response is heavily influenced by the chemistry of water-soluble compounds (Van der Putten, *et al*., 2001).

In addition to induced changes in leaf chemistry, several defense compounds are manufactured in the root region and have the potential to leach into surrounding soils (Van der Putten, *et al*., 2001), and above-ground herbivory has been shown to reduce nutrient availability to root-feeding insects (Van der Putten, *et al*., 2001). Defense

compounds also impact the structure of insect communities by affecting host preference during colonization by influencing local soil conditions (Inui, Miyamoto and Ongushi, 2003). By examining soil invertebrate populations under damaged and undamaged plants, we can gain additional insight into the secondary effects of induced defense responses in an ecosystem.

C. Study Rationale

C.1. Gaps in Plant Defense Research

Herbivory has frequently been investigated by crop scientists in efforts to estimate the economic costs of insect predation (Strauss and Agrawal, 1999). Early studies often focused on individual plants and gave little attention to the role that insects play in dictating plant assemblages, primarily due to the assumption that insects were not food-limited and that the damage to natural plant communities due to herbivory was negligable (Gange, 1990). In the past few decades this view has been challenged and the impacts of predation more closely examined (Gange, 1990).

There remains some dissagreement on the magnitude of the effect that herbivory has on ecosystems (Gange, 1990). This may be due to the fact that few field studies get fully repeated, which may limit results because herbivory often varies with factors such as timing, season, life stage, weather, and other factors that may confound shorter or more limited studies (Gange, 1990). It has also been discovered that environmental factors play a considerable role in the outcome of any plant-herbivore relationship, and variations in plant competition (Cottam, Whittaker and Malloch 1986), external stress (White, 1984), and surrounding vegetation (Parker and Root, 1981) can affect the types

and abundance of plant-insect interactions. Rarely does research on herbivory extend beyond plant and herbivore populations to the level of broader ecosystem function, such as nutrient cycling and decomposition (Harper, 1990).

C.2. A Novel Holistic Approach to Plant Defense Research

Studies have displayed positive feedbacks among soil quality, litter composition, and nutrient release (Enoki and Kawaguchi, 2000), and have also indicated a relationship between the quantity and composition of soils and the organization of soil invertebrate communities (Hasegawa, 2001). However, research investigating a direct link between plant chemistry and invertebrate community has been limited up to this point and may provide missing informational pieces to the complex puzzle of plant-herbivore relationships.

These gaps warrant further research into the secondary effects of herbivory. A change in any aspect of an ecosystem will often cause a cascade of modifications in the local ecosystem. Therefore, fluctuations in leaf composition, as occcurs with wounding, are expected to cause changes in the soil, and its inhabitants. By examining the relationship between plants, soil, and detritivore invertebrates, my aim was to identify secondary consequences of herbivory. To investigate these relationships, I assessed the soil invertebrate abundance and species richness under both damaged and undamaged plants of three shrub species in the Albany Pine Bush Preserve. I also measured growth and post-treatment browsing in an effort to detect possible whole-plant responses. I predicted that if damaging plants results in a change of chemical composition in the leaves, then leaf litter quality would be altered, leading to changes in the invertebrate communities that feed on leaf litter. Therefore, it was hypothesized that if plant defense

compounds have secondary effects on soil communities, then soil invertebrate abundance and species richness would vary significantly between damaged and undamaged plants, and that variation between treatment groups would be more robust than differences among plant species or soil pH.

2. STUDY AREA AND METHODS

A. Study Site and Test Subjects

A.1. Albany Pine Bush Preserve

The Albany Pine Bush Preserve is a rare inland pine barrens ecosystem that was formed as glaciers receded 12-15 thousand years ago (APB, 2010). The cooperative efforts of the Albany Pine Bush Preserve Commission (APB, 2010), The Nature Conservancy, local municipalities, and concerned citizens have protected about 3,100 acres of this unique ecosystem, in an effort to conserve biodiversity as well as promote education and research about inland pine barrens (APB, 2010). The APBPC also undertakes management programs to preserve the characteristics and unique habitats of the pine barrens, including prescribed burnings, herbicide application, mowing, and propagation of native plants (APB, 2010). Many rare and threatened species make their home in the APB, including the endangered Karner Blue butterfly (*Lycaeides Melissa samuelis*), whose obligatory host plant is the rare blue lupine (*Lupinus perennis*). Promoting the growth of blue lupine through interseeding and maintenance of open, grassland habitats is one of the management goals of the APB Preserve Commission.

My work was conducted in a mixed shrub community in the Albany Pine Bush Preserve, under a research permit issued to Dr. Robinson. Three native shrub species were used in this study, dune willow (*Salix pumila*), dwarf chestnut oak (*Quercus prinoides),* and scrub oak (*Quercus ilicifolia*), chosen due to their relative abundance, shared habitat, and relative ease of measurement and manipulation. They also exhibit well-documented leaf defense chemistry.

A.2. Shrub Species Examined

A.2.a. *Salix humilis* var. *tristis* (Ait.) Griggs (dune willow)

The dune willow is found on dry or barrens soils often as a component of early successional prairie. It is native to NE North America, and is most likely to occupy dry black soil prairies, sand prairies, sandy savannas, barrens, and gravelly steeps (USDA, 2010). Dune willow is small (approximately 1 m tall) with dull, hairy, untoothed leaves (Barnes, 2003) (Figure 1A). It is found in the pitch pine-scrub oak barrens of the Albany Pine Bush, and flowers in late March and early April. These shrubs sprout new stems and proliferate in response to fire. The dune willow is a host plant for viceroy caterpillars and the larvae of a gall midge in the genus *Rhabdophaga* (Barnes, 2003).

A.2.b. *Quercus prinoides* Willd. (dwarf chestnut oak)

Dwarf chestnut oak populations are found in pockets of poor, dry spoils in E. North America. They are often found dominating pitch pine-scrub oak communities of inland barrens such as the Albany Pine Bush (Barnes, 2003). Dwarf chestnut oak is a member of the white oak group, growing to 60- 305 cm tall with small lobed leaves that terminate in sharp points (Figure 1B). It flowers in May and produces acorns that mature in the same year. These shrubs are known for their impressive ability to recover from intense fires, increasing acorn production after the burning eliminates diseased and insect-infested old growth. They are subject to predation by the inland barrens buck moth and may host oak fig galls (Barnes, 2003).

A.2.c. *Quercus ilicifolia* Wang. (scrub oak/ bear oak)

Scrub oak thrives on the poorest of dry, acidic soils with recent major disturbances, such as fires. It is distributed throughout the eastern United States as an

example of an Atlantic Coastal Plain species whose range expansion is probably due to postglacial warming (Barnes, 2003). Populations form dense thickets and are intolerant of shade. Individuals may be as tall as 6 m, with dark green, broad, pointed leaves that end in toothed lobes separated by shallow sinuses (Barnes, 2003) (Figure 1C). The stems, which arise from a large, gnarled crown with numerous strong, descending roots, recover quickly from fires and may grow to half the original size during the first growing season after fire or cutting (Barnes, 2003). Mature scrub oaks produce acorns in late April to mid-May (Barnes, 2003). This shrub is often host to a suite of gall-making wasps and numerous leaf-eating caterpillars and larvae (Barnes, 2003).

Figure 1. Photographs of dune willow (A) (taken at site in August 2010), chestnut oak (B) (photo by Ricketts Glen, 2008) and scrub oak (C) (taken at site in August 2010)

A.3. Study Site Management and Specifics

All of these test subjects are located in the 'Alley Cat' and 'Andromeda' management parcels of the King's Road Barrens in the Albany Pine Bush Preserve, Albany, NY (42°308'N, 18°592030'W) (Gifford, N., personal communication). Figure 2 shows a photograph of this area, and GIS coordinates of all plant subjects are listed in Appendix 1. In 1988, New York State Legislature charged the APBPC with maintaining the fragile qualities of the Pine Bush with periodic coordinated management including controlled fires, herbicide application, and mowing. Since then, this area has undergone intense management by the Albany Pine Bush Commission to maintain an open landscape.

As described by Neil Gifford, APBPC Conservation Director (personal communication), 'Alley Cat' has been heavily managed, including some of the most intense of controlled fires observed in the Pine Bush. This 1.6 hectare area was mowed in June 2003, then burned in July 2005. It was mowed again in June 2008, and in August 2008 herbicide was applied to approximately 35% of the existing scrub oak plants (Gifford, N., personal communication). Complete removal of above-ground plant material and intense burning of the landscape resulted in a depletion of accumulated organic matter, including the humus layer of the soils.

"Andromeda" has also undergone intensive management. This parcel was mowed in June 2004, and then burned in July of the same year (Gifford, N., personal communication). Blue Lupine was interseeded in July 2004, subsequent to subscribed burning, in an effort to produce habitat for Karner Blue Butterfly populations. The area

was then mowed in June 2008, and herbicide was applied to approximately 35% of the scrub oak population in August 2008.

Soils in both parcels of interest in the ABP are young, and consist mainly of Colonie Loamy Fine Sand that is very deep, well-drained, acidic, and course-textured (APB, 2010). This dry, sandy, acidic soil does not promote decomposition, and much of the nitrogen in organic matter is volatilized and lost (APB,2010) resulting in relatively poor soil conditions low in nutrient content.

Figure 2. Photographs of the study site on King's Barrens Road in the Albany Pine Bush Preserve (taken August, 2010)

B. Treatment

My primary experimental tool for this study was clipping green leaves, which simulates naturally-occurring damage by leaf-eating organisms (Shiojiri and Karban, 2008). Plants of three shrub species were identified, tagged, measured, and designated as either control or treatment in July 2009 (N=90, 30 plants per species). Treatment was administered through leaf cutting with shears to result in 25 percent damage to the peripheral plant mass, with a focus on removing leaves. Size and diameter of main stem were assessed prior to clipping15 plants of each species, treatment administered for two consecutive years. Fifteen plants of each species were left untreated as a control group. Initial treatment was completed in August of 2009, with a second treatment administered to the same treatment group in June 2010.

C. Assessment of the Effects of Stress-Induced Plant Responses

C.1. Leaf Chemistry

Following treatment, leaf specimens were collected from all 30 plants per species and analyzed for Carbon/Nitrogen concentration on a seasonal basis (spring, summer, fall) to detect any changes that resulted from the induced stress of leaf cutting. Leaves were collected from various parts of the plant, targeting new leaves, with 10 being taken from each prairie willow and five from both the scrub oak and chestnut oak plant. All leaf samples were bagged separately and placed in a drying oven (60° C) for two days prior to shipment to Dr. Kaori Shiojiri of the Center for Ecological Research, Kyoto University, Japan. The dried leaves were then ground to a powder with a grinder (IFM-600D, Iwatani

Co. Japan), and N and C concentrations were measured using an elemental analyzer (JM 1000 CN, J-Science Co. Ltd, Kyoto, Japan).

C.2. Soil Invertebrate Community

Soil samples were also taken from twenty plants of each species, ten controls and ten treated, during July 2010, in order to compare local soil invertebrate communities. Samples were taken approximated 12 cm away from main stems, to a depth of 10 cm. Total volume was approximately 68-70 cm^3, however mass was uneven due to variation in substrate density and composition. Total wet weight was recorded for each sample before placement in Berlese funnel extractors (N=60). Samples were placed 10-12 cm beneath 30 watt bulbs for 48-50 hours, during which time invertebrates are collected in a solution of 80% ethanol. Invertebrates were identified to order or family using taxonomic manuals.

C.3. Soil pH

In addition, soil pH was assessed for each soil sample taken (N=60). Soil samples were sifted to remove all organic matter, after which they were mixed with distilled water. Twenty-five grams of sifted soil were added to 25 ml of distilled water and mixed thoroughly for approximately 30 seconds. Solutions then sat undisturbed for 15 minutes, after which the pH was taken from the top aqueous layer of the soil mixture, using a dedicated soil probe connected to an *Accumet* model 20 pH/conductivity meter at room temperature (21-23°C). The pH meter was calibrated every 3 days to ensure accuracy. These measurements were then used to create a pH contour map of the study area.

C.4. Plant Growth and Tests for Post-Treatment Browsing

The effects of cutting on plant growth were also examined in the following year after treatment (June 2011). Inter-nodal lengths, indicating the rate of branch extension, are a common measure of growth in woody plants. This was examined to detect any differences in the rate of growth between treatment and control groups. The previous year's growth, including length and diameter, were taken from five live branches of each plant one year after treatment (N=445). This study area contains populations of white-tailed deer (*Odocoileus virginianus*) and other browsers. Post-treatment browsing of stems was assessed by subsampling stems of all 90 plants to determine the ratio of browsed branches versus those with no evident damage.

D. Analytical Methods

Statistical analysis of invertebrate populations was completed utilizing the statistical software package JMP 9.0. Contingency testing was completed to assess the variability of C:N of plant samples between species, sample period and treatment groups. 2-way ANOVA tests were run to compare soil invertebrate species richness (the amount of different taxa represented) and abundance (the total amount of organisms found) in the three plant species, control versus treated plants collectively, and controlled versus treatment for each plant species. In addition, relative abundance curves were assessed for all soil samples, pooled by treatment. 2-way ANOVA tests were also run to test for relationships between soil invertebrate diversity and pH. Surfer 7.0 was used to create a contour map of pH values in the study site. New plant growth was analyzed using

ANOVA tests, while contingency tables with Chi-square analysis compared browsing data between treatment and control groups for the three plant species.

3. RESULTS

A. Leaf C:N

Variation in nutrient (C:N) ratio was greater among plant species and sampling period than between treatment groups (Table I and II). No significant difference was observed in the C:N analysis of treatment versus control groups when all plants and periods were grouped (Table I, Figure 3). In contrast, C:N ratio varied greatly among both species and sample periods (Table I, Figure 3). C:N ratio was lowest in dwarf chestnut oak and highest in scrub oak (Figure3).

Table I: ANOVA table for the effect of plant species and treatment group on C:N analysis of leaf samples for all period combined

Source of Variation	df	Mean Square	*F*	P
Plant species	2	1413.826	25.990	0.000
Treatment group	1	3.442	0.063	0.802
Species ˣ treatment	2	41.340	0.760	0.468
Error	440	54.400		

C:N varied seasonally (Table II), as depicted in Figure 3. Mean C:N values were just above 20.5 for periods 1 and 2 (before treatment and after treatment, spring 2010), but then spiked sharply in early summer (July 2010), before dropping dramatically in late summer (Aug 2010) and recovering somewhat in the fall (Oct 2010).

Table II. ANOVA table for the effects of treatment group and sampling period on C:N analysis of leaf samples for all three plant species combined

Source of Variation	df	Mean Square	*F*	P
Treatment group	1	5.712	0.240	0.625
Sample Period	4	4099.477	171.982	0.000
Treatment × period	4	10.421	0.437	0.782
Error	436	23.837		

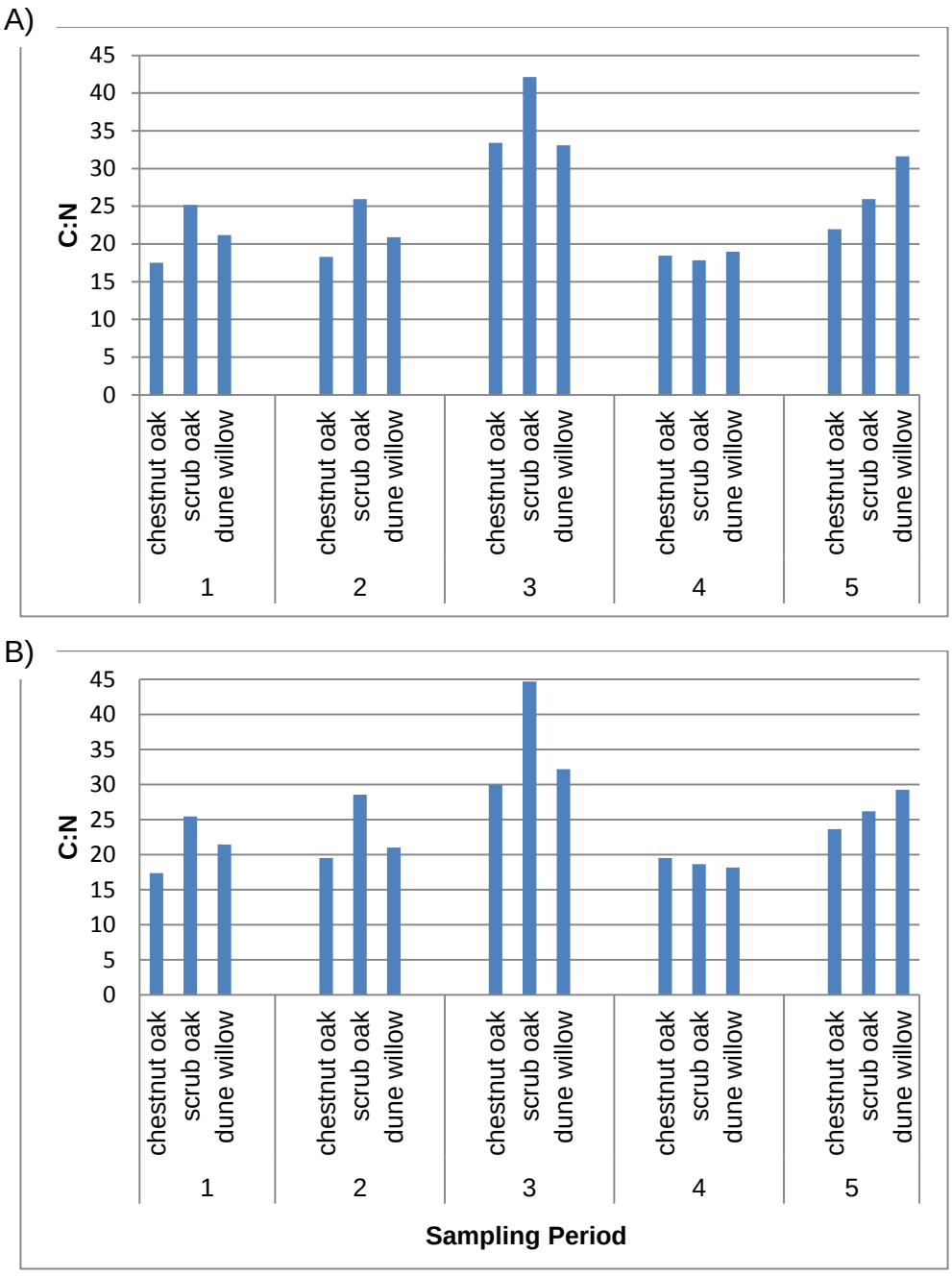

Figure 3. Bar graphs depicting mean C:N for each plant species in each sampling period for control samples (*A*) and treatment samples (*B*). Sampling periods are as follows: 1= prior to treatment (May 2010), 2= late spring (June 2010), 3= early summer (July 2010), 4= late summer (Aug 2010), 5= fall (Oct 2010)

B. Soil Invertebrate Communities

Differences were found in the composition and abundance of soil invertebrates between treated and control groups. Appendix B reports all invertebrates identified in control and treated sample collections (N=482). As shown, 349 invertebrates were identified from control samples, while 133 were from treated plants. Control groups had 2.62 times the abundance of invertebrates in their surrounding soils than did treated groups. Richness was also lower in treated groups, with 15 taxa represented as opposed to 20 in control groups. Figure 4 compares the relative abundance of the top ten represented soil invertebrate groups to indicate how evenly distributed taxa are in each group. Dominance seems somewhat higher for control groups whose slope is closer to a geometric pattern.

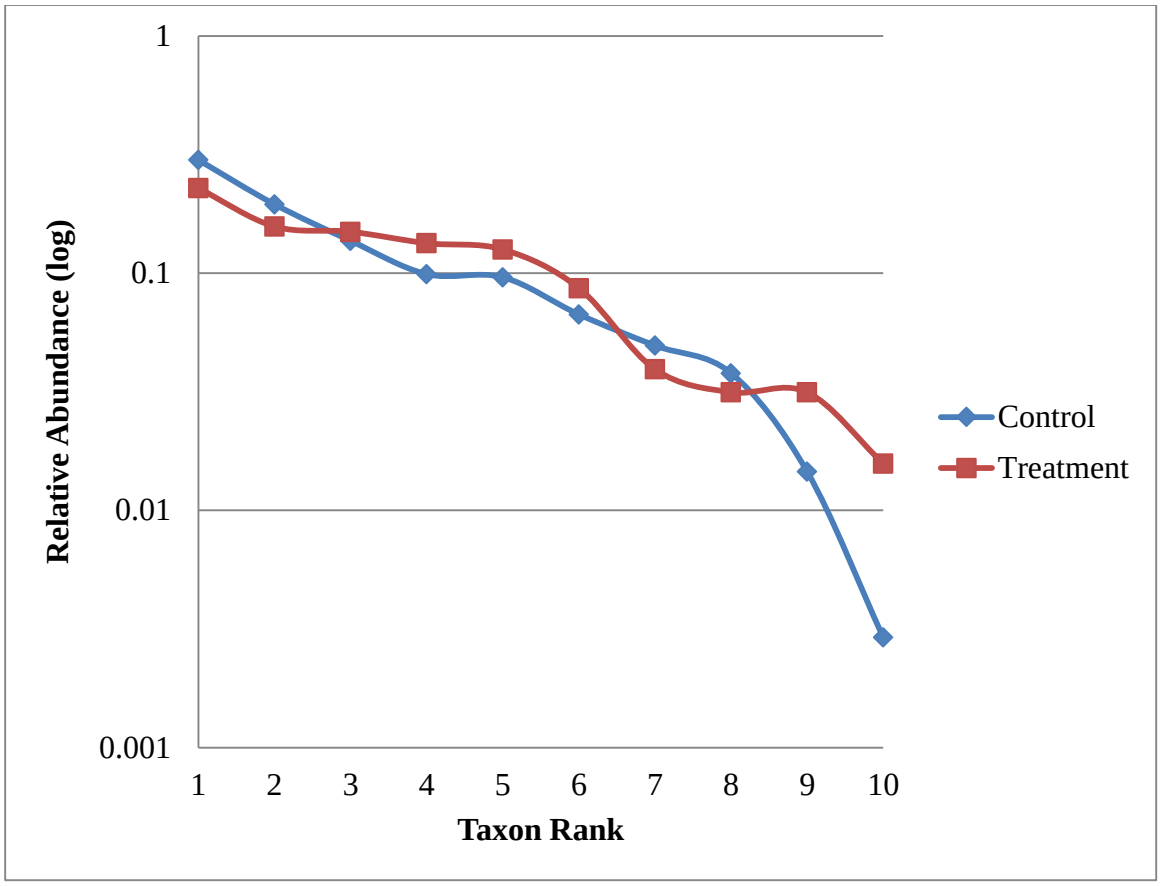

Figure 4. Relative abundance of top ten represented soil invertebrate groups for control and treatment groups of invertebrate data, pooled for all species in control and treatment groups. Slope of logarithmic trend line is -0.09 for treatment, -0.124 for control.

Results indicate significant difference in soil invertebrate abundance between treatment and control groups. Differences were greater among treatment groups than among plant species (Table III).

Table III. ANOVA table for the effects of treatment group and plant species on soil invertebrate abundance

Source of Variation	df	Mean Square	*F*	P
Plant species	2	156.217	3.632	0.033
Treatment group	1	707.267	16.445	0.000
Species x treatment	2	7.317	0.170	0.844
Error	54	43.007		

Results also indicate significant differences in species richness between treatment and control groups. Again, these differences were greater among treatment groups than among plant species (Table IV).

Table IV. ANOVA table for the effects of treatment group and plant species on soil invertebrate richness

Source of Variation	Df	Mean Square	*F*	P
Plant species	2.0	9.517	2.686	0.077
Treatment group	1.0	70.417	19.877	0.000
Species x treatment	2.0	6.717	1.896	0.160
Error	191.300	54	3.543	

Figure 5 displays these trends as the mean species richness and abundance for each treatment group plotted by plant species. Soil invertebrate communities were more abundant under control willow plants than under treated plants (post-treatment soil invertebrate abundance mean ± 1 SE: control 8.3 ± 4.9, treated plants 1.1 ± 1.3; post-treatment soil invertebrate species richness mean ± 1 SE: control plants 4.2 ± 1.3, treated plants 0.9 ± 1.1). Similarly, soil invertebrate abundance and richness was higher in samples taken under control dwarf chestnut plants than under experimentally clipped plants (post-treatment soil invertebrate abundance post-treatment soil invertebrate abundance mean ± 1 SE: control 13.6 ± 8.4, treated plants 5.8 ± 7.9; post-treatment soil invertebrate species richness mean ± 1 SE: control plants 4.3 ± 2.3, treated plants 3.1 ± 1.5). Scrub oak samples also showed a similar pattern (post-treatment soil invertebrate abundance mean ± 1 SE: control 18.8 ± 8.0, treated plants 6.4 ± 6.9; post-treatment soil invertebrate species richness mean ± 1 SE: control plants 5 ± 2.7, treated plants 2.8 ± 1.8). ANOVA results of comparison of soil invertebrate abundance in between treatment groups for each plant species is shown in Table V, and comparisons of species richness is reported in Table VI. As shown, soil invertebrate abundance was significantly lower among treatment samples for willow and chestnut oak samples (Table V), while richness was lower among treated willow and scrub oak (Table VI).

Tukey-Kramer pairwise analyses were completed to compare the three plant species. Results indicated no significant difference in total abundance between test species; however, species richness varied significantly in dune willow samples, which were significantly lower than those for oak species (Figure 5).

Table V. ANOVA results of total soil invertebrate abundance of each plant species

Plant Species	Source	df	Mean Square	*F*	*P*
Willow	treatment	1	259.2	19.8536	0.0003
Error	treatment	18	235	13.056	
Scrub Oak	treatment	1	204.8	3.586	0.0745
Error	treatment	18	1028	57.11	
Chestnut oak	treatment	1	304.2	4.5178	0.0476
Error	treatment	18	1212	67.333	

Table VI. ANOVA results of soil invertebrate species richness of each plant species

Plant Species	Source	Df	Mean Square	*F*	*P*
Willow	treatment	1	54.45	36.98	<0.0001
Error	treatment	18	26.5	1.47	
Scrub Oak	treatment	1	24.2	4.5565	0.0468
Error	treatment	18	95.6	5.3111	
Chestnut oak	treatment	1	7.2	1.8783	0.1874
Error	treatment	18	69	3.83333	

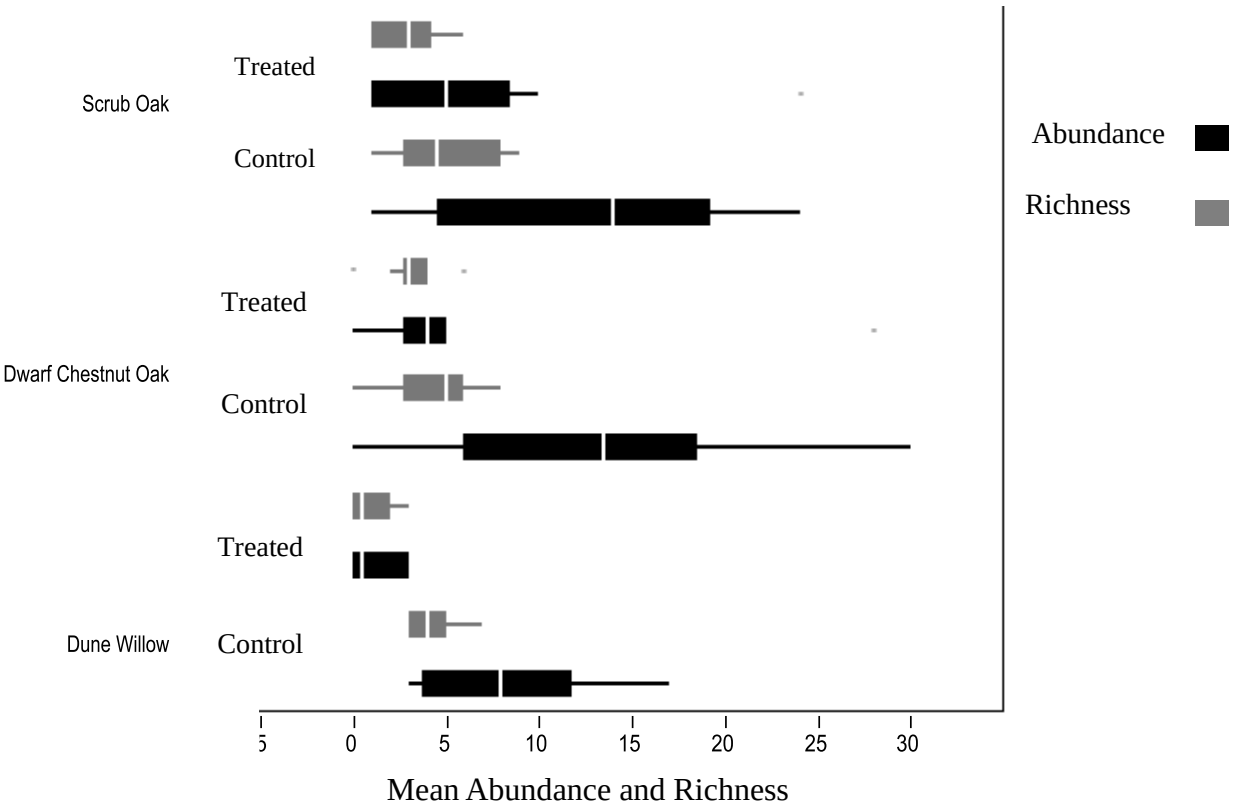

Figure 5. Boxplot comparison of mean soil invertebrate species abundance and richness for treatment and control groups of three plant species in the Albany Pine Bush.

C. Soil pH

Analysis of soil pH revealed acidic soils under all plant specimens. A contour map, shown in Figure 6, depicts the patterns of acidity in this study area of the Kings Road Barrens area in the Albany Pine Bush. The range of pH was from 3.3 to 5.2, with an average pH for all three plant species, both pooled and individually, of 4.2.

ANOVA analysis revealed no significant relationship between soil invertebrate abundance and soil pH (Table VII). However, soil invertebrate species richness was significantly affected by soil pH (Table VIII), indicating a reduction in the amount of soil invertebrate taxa represented when soil conditions were at the extreme ends of soil pH ranges (post-treatment pH mean $\pm$ 1 SE: 4.18 $\pm$ 0.40) .

Table VII. ANOVA table of the effects of treatment and soil pH on soil invertebrate abundance

Source of Variation	df	Mean Square	*F*	P
Treatment group	1	738.335	16.074	0.000
Soil pH	1	31.164	0.678	0.414
Error	57	45.935		

Table VIII. ANOVA table of the effects of treatment and soil pH on soil invertebrate species richness

Source of Variation	df	Mean Square	*F*	P
Treatment Group	1	82.024	22.710	0.000
Soil pH	1	17.897	4.955	0.030
Error	57	3.612		

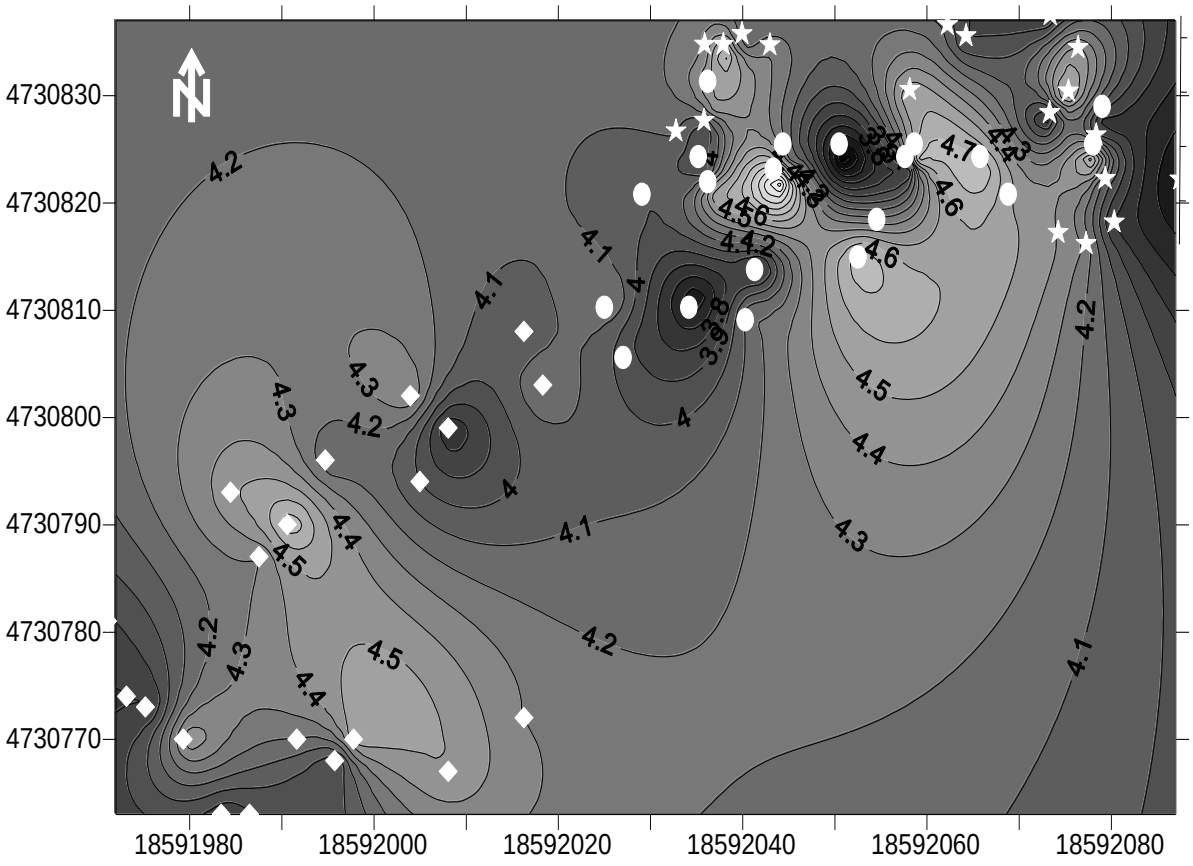

Figure 6. pH contour map of the study area of interest in the Albany Pine Bush Preserve. Diamonds indicate the location of individual dwarf chestnut oak test subjects, circles indicate scrub oak, and stars indicate dune willow.

D. Plant Growth

Internodal length and diameter did not appear to be affected by treatment, as less than 0 percent change was observed between treatment (mean ± 1 SE: intermodal length 18.71 ± 7.52, diameter 2.57 ± 1.13) and control (mean ± 1 SE: intermodal length 19.18 ± 7.76, diameter 2.64 ± 1.18) groups for all three plant species (Appendix C). Growth did vary greatly among dune willow (mean ± 1 SE: intermodal length 23.85 ± 6.29, diameter 1.48 ± 0.42) , scrub oak (mean ± 1 SE: intermodal length 18.79 ± 7.72, diameter 3.66 ± 0.96), and chestnut oak (mean ± 1 SE: intermodal length 14.51 ± 5.72, diameter 2.65 ± 0.73); however, these differences are expected due to natural morphological variation.

E. Post-Treatment Browsing

The percentage of browsed versus not browsed of all branches assessed for treatment and control groups of each test subject species are shown in Figure 6. Pearson Chi- Square analysis revealed significant differences between the ratio of browsed and not browsed branches on dune willow test subjects (Table VII).

Table IX. Table of contingency test results for the ratio of browsed and not browsed branches of treatment versus control groups of three shrub species

Plant Species	Test Statistic	Value	df	*P*
Dune willow	Likelihood ratio Chi-square	15.101	1	0.000
Scrub oak	Likelihood ratio Chi-square	6.159	1	0.013
Chestnut oak	Likelihood ratio Chi-square	0.307	1	0.580

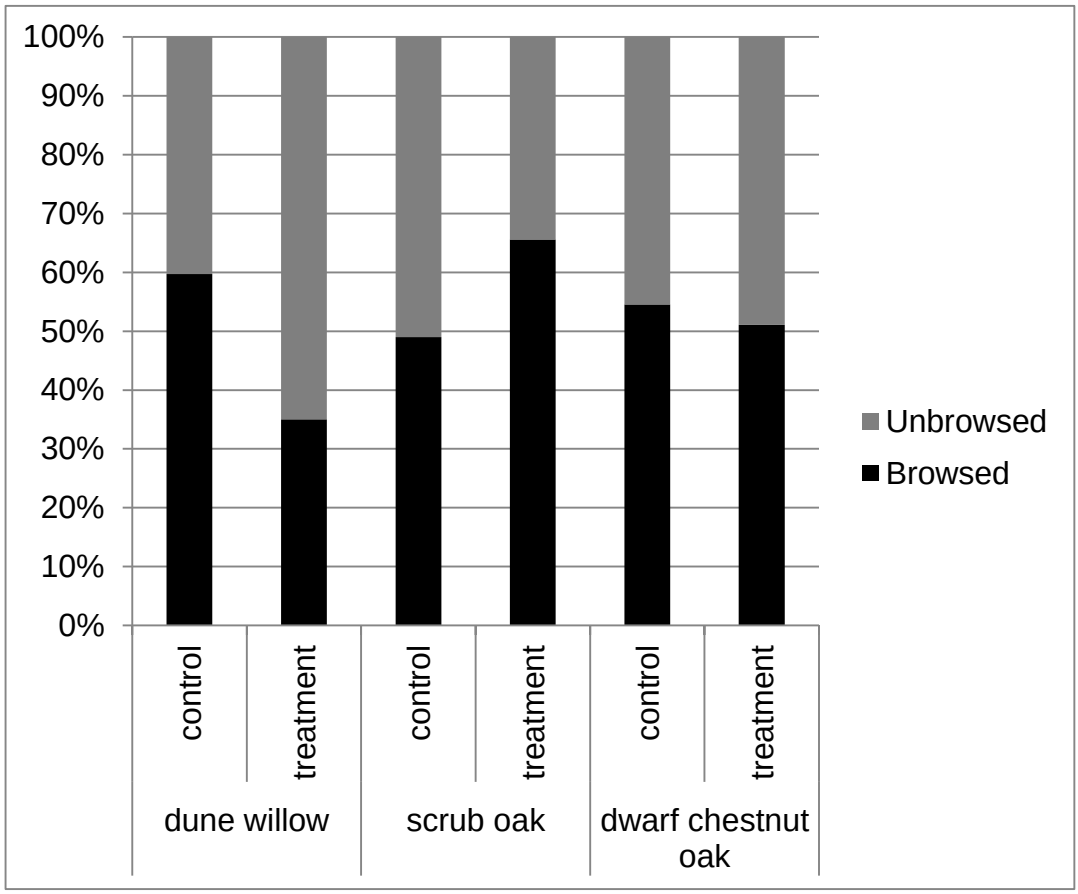

Figure 7. Bar graph representing the proportion of browsed versus unbrowsed branches for treatment and control test subjects of three plant species

4. DISCUSSION

A. Results of the Secondary Effects of Plant Defense Induction- Summary

Results from this experiment indicate decreased soil invertebrate species richness and abundance under treated plants. Experimental clipping of plants has been shown to cause the release of an epimer of methyl jasmonate, an induced volatile compound that triggers production of defensive compounds (Karban, *et al.,* 2000). This study employed similar techniques in a natural shrub community in the Albany Pine Bush, after which assessments were taken to determine the secondary consequences of stress induction. This study had the novel approach of investigating the cascade of affects from induced plant responses through an examination of both the above and below ground effects of mechanical damage. Understanding the details of these interactions can lead to greater insight in chemical ecology.

Chemical assay of clipped branches in sagebrush revealed a 10.8-fold increase in MeJA volatile concentration subsequent to clipping (Karban, *et al.,* 2000). This is one of many studies that show that manual cutting induces defense mechanisms. Such changes should be reflected in the leaves, and, because leaf litter is a vital and dynamic component of soil, changes in leaf chemistry were expected to cause alterations in soil composition and corresponding invertebrate populations. The general results of this experiment indicate a significant difference in both species richness and abundance of soil invertebrates between treatment and control groups, supporting the hypothesis that variations in leaf chemistry caused by mechanical damage will result in differences in soil invertebrate communities.

Contrary to predictions, leaf chemistry did not vary accordingly, however only C:N was analyzed. No significant differences were found in the whole plant growth rates of treated and control groups, but I found some differences in the ratio of browsed and unbrowsed branches among willow plants. Soil pH analysis implied overall acidic conditions in this study site, even though it had been recently burned by the Albany Pine Bush Commission staff. Burned sites typically contain more basic soils than unburned sites in pine barrens (Jha, 2008), and the observed low pH indicates a relatively quick recovery from the effects of prescribed management burning, and a return to the normally acidic conditions characteristic of pine barren ecosystems.. In the following paragraphs, the limitations and results of these findings are interpreted and related.

B. Effects of Induced Plant Defense Compounds on Soil Invertebrate Communities

The majority of soil invertebrates identified in all samples are defined as mesofauna, intermediate-sized animals 0.1 to 2mm. Much of the variation observed is due to differences in soil Acari, many of which function to facilitate the decomposition process and aid in nutrient cycling to increase soil fertility and structure (Gope and Ray, 2006). Mites are known to feed on microflora, including fungi and bacteria, thereby influencing soil microbial dynamics (Gope and Ray, 2006). Collembola abundances were different between treatment groups. They are known to be fungal feeders, whose spatial distribution is closely associated with the distribution of mycelia and spores (Neher, 1998). These two groups accounted for most of the differences in soil faunal abundance. The ecological niche of mesofauna is broad and they occupy all trophic levels, feeding on plants, microbes, animals, and detritus (Neher,1998). While macrofauna, such as adult

Hymenopterans, directly aerate and mix the soil through burrowing and tunneling activities, mesofauna tend to impact soil communities through altering the microbial community, which in turn effects decomposition (Neher, 1998). Collembolans and mites may enhance microbial activity, accelerating decomposition and mediating important transport processes in the soil (Neher, 1998). The dependencies between mesofauna and microfauna suggest that treatment differences may be difficult to interpret without additional study.

Plants affect soil biota in other ways, such as through shading, soil penetration, and various interactions at the root zone. It is claimed that soil processes and the communities that govern them are influenced primarily by climate, followed by edaphic factors, then the quality of plant material, and finally by innate biological systems (Lavelle, 1996). To clarify the relationship between plants and soil invertebrates, it is important to address how all these factors are altered by plant stress imposed by herbivory. For example, the removal of above-ground plant material, which occurs both in this study and through intense herbivory, may reduce the amount of shading given by plants, and may account for some of the decrease observed in soil communities following treatment. Closer examination of these factors may help identify the differences in biodiversity and abundance as observed in this study.

However, it is possible that soil invertebrates or their food sources are responding directly to chemical changes within the plant leaves or roots. White (1984) suggested that extreme damage nearly always made plants more unfavorable as a food source for herbivores, while slight damage has the potential to relocate N concentrations within the plant and make it more favorable for developing insects (White, 1984). The effect of

>25% removal of leaves may be considered ‘extreme’ and may result in creating a less favorable food source for invertebrates.

Regardless of the direct cause, a decrease in soil invertebrates has compounding effects on the greater ecosystem. Biodiversity allows an ecosystem to maintain constancy of function through fluctuating environmental conditions (Neher, 1998), which is particularly important for a foundational component such as soil. Invertebrate communities may be useful indicators of the quality of soil systems, since they are generally very sensitive to perturbation (Lavelle, 1996). The functional importance of soil invertebrates can be disproportionate to their actual abundance (Anderson, 1988), emphasizing the significance of diversity in relation to soil resilience (Lavelle, 1996). This is of particular concern in agricultural lands as evident in the possible links between the reduction of soil regulators and the lack of sustainability in large scale agricultural systems (Lavelle, 1996).

For unexplained reasons, the number of taxa decreased as soil pH moved away from average. This indicates that the optimum range within the range of pH tolerance for most soil invertebrates around the average pH reading, 4.1. It is expected that most organisms will be found in the mesotypic range of any environmental gradient. However, apparent treatment effects were notably stronger.

C. Leaf C:N Analysis

Contrary to predictions, no significant difference was observed between the C:N ratio of treatment and control plants. However, there may be additional factors to consider that would make this finding inconclusive. One explanation may be that soil invertebrate

communities are more affected by interaction with roots, where defense compounds are often produced before being transported to the rest of the plant (Ven der Putten, *et al*., 2001). Compounds may leach into the surrounding soil and effect the detritus community directly. Another possible explanation relates to an important property of the leaves themselves.

I assumed that the primary detrital input to the sampled soils was primarily fallen leaf litter located in the immediate area of each plant. An important precursor to the shedding of leaves is nutrient retranslocation through which certain nutrients are mobilized away from senescing leaves and deposited into other parts of the plant (Aerts, 1996) , which allows for critical nutrients to be conserved and re-used (Chapin, 1980). Therefore, any induced chemicals remaining in the litter were likely to be insoluble compounds that are not re-mobilized for translocation.

Deciduous trees and shrubs, such as the species of concern, retranslocate significantly more N prior to shedding than evergreens, with an average resorption efficiency of 54% (Aerts, 1996). Seasonal concentrations of Nitrogen, Potassium and Phosphorous in the plant can vary significantly when these compounds are retranslocated (Saur, Nambiar, and Fife, 2000) while carbon based compounds, such as cellulose, lignins, and tannins, remain in the shed leaves. This practice of retranslocation may render C:N analysis of leaf litter inconclusive, because fallen leaves do not depict the actually nutrient composition present within the plant and may not accurately capture the full effect of stress induced responses on nutrient ratio within leaves. Additional testing would be necessary to eliminate this confounding variable and clarify the changes stress may cause within living, non-senescing leaves.

However, the data collected during this study did provide interesting results. It is clear that the nutrient ratio varies significantly among plant species. This is in addition to natural variation, often caused by spatial small-scale soil heterogeneity that tends to be emphasized in drier soils and can greatly impact plant biology (Hook, Burke, and Luenroth, 1991). Defense compound production may also account for variation in C:N, since each plant species may develop a specific suite of defense compounds. For example, many defense compounds, such as phenols, terpines, and sulfur and nitrogen compounds are derived from secondary metabolites that are very restricted in distribution and primarily found only in one plant species or related group of species (Mazid, Kahn and Mohammad, 2011). Variation in C:N is expected due to both the immediate environment and unique characteristics of each plant and plant species, including differences in defense strategy.

An additional finding of the C:N analysis supports the existence of seasonal nutrient variation within leaves. C:N spiked in third period samples, which were taken mid-summer during the hottest, driest part of the year. The ratio subsequently dropped dramatically in early fall, before recovering somewhat late fall. A study that compared leaf nutrient content in numerous grass species found evidence that leaf physiological status varies annually, and that the significant variation among species' seasonal patterns may be attributed to species-specific responses (Enriquez, Marba and Cebrian, 2004).

D. Effects of Treatment on Plant Growth and Post-Treatment Browsing

Results from whole plant growth analysis indicate no significant difference between treatment and control groups. According to McNaughton (1983), there are three

theories regarding the effects of herbivory on plant fitness. The first claims that herbivory is always detrimental to the plant eaten; second, plants can compensate for low levels of herbivory, resulting in no net change in fitness; and third, moderate levels of herbivory may actually cause a compensatory response through which fitness is increased (McNaughton, 1983). McNaughton included both vegetative tissue growth and reproductive success in his definition of fitness, and believed that an increase in these indices following herbivore damage was indicative of a compensatory response developed through evolutionary responses. The whole plant growth results from this study more closely align with the first (and more commonly held) theory that herbivory decreases plant fitness. However, investigation into the differences in reproductive rates may provide additional information regarding a plant's compensatory abilities. This would be an interesting and worthwhile future addendum to this study.

Analysis of the frequency of browsed and non-browsed branches on plant subjects suggested that treated (clipped) dune willow plants were browsed more than expected, but browsing was less than expected in scrub oak . These findings are inconclusive due to the possible impacts of seasonal variability in the browsing behavior of major herbivores in the study area because of differences in times of assessment. However, it is known that many defense compounds cause a plant to be toxic or less palatable, which may infer increased resistance among scrub oak subsequent to treatment.

E. Additional Information

The results from this research are intriguing, but preliminary, and require additional assessments to confirm and further depict the cascade of reactions due to stress-induced

plant responses. Repeating this trial in a variety of ecosystems with numerous test species would aid in clarifying and establishing general trends. Nevertheless, it is clear from these results that ecosystems are not the sum of isolated components, and interactions can be passed through different levels of ecological organization.

It is important to keep in mind that this study site has been heavily managed through mowing, prescribed burning, and herbicide application (Gifford, personal communication). Extremely hot fires completely removed the organic layer of the soil, creating a young (< 6 years) soil profile with relatively new invertebrate populations (Gifford, personal communication). In addition, most dune willow plants in this study were located within a frost pocket, where cold air collects after draining from higher levels (Gifford, personal communication). In addition, careful selection of plants to control for microclimate differences, such as frost pockets, should be considered prior to additional investigation. It is possible that cooler air and soil temperatures play a role in shaping soil invertebrate communities, an additional explanation for the differences observed in dune willow as compared to the oak species.

F. Management to Preserve Rare Ecosystems for Research

This study could not have been conducted without the management efforts of the Albany Pine Bush Preserve Commission. Through prescribed fires, mowing, and herbicide application, management maintains the unique qualities of a pitch pine- scrub oak barrens and prevents succession towards old growth forests. All shrubs examined within this study are dependent on the existence of dry, sandy substrate and low tree canopy cover for their populations to thrive. Because of the suppression of natural fires

and disturbances, controlled burnings are necessary to prevent secondary succession and the accumulation of organic matter. The experimental conditions are similar to the more arid environments, where similar work has been conducted. The low scrub vegetation and nutrient-poor soils offer a more simplified and tractable system than other regional ecosystems.

The Albany Pine Bush Preserve Commission uses a holistic, ecosystem based approach to its management practices, taking into account the connections between above ground vegetation and wildlife and the soil ecosystems that support it. Preserving natural systems requires protecting fragile components- not only rare species, but the processes that promote heterogeneous vegetation (Christensen, 1996), such as the unusual shrubland where my work took place.

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APPENDIX A

UTM coordinates for shrub test subjects

Plant ID Number	Plant Species	UTM (Easterly)	UTM (Northerly)
301	dune willow	18592033	4730826
302	dune willow	18592036	4730827
303	dune willow	18592036	4730830
304	dune willow	18592036	4730831
305	dune willow	18592036	4730834
306	dune willow	18592038	4730834
307	dune willow	18592040	4730835
308	dune willow	18592043	4730834
309	dune willow	18592040	4730835
310	dune willow	18592058	4730830
311	dune willow	18592062	4730836
312	dune willow	18592058	4730835
313	dune willow	18592064	4730835
314	dune willow	18592065	4730833
315	dune willow	18592073	4730831
316	dune willow	18592073	4730828
317	dune willow	18592075	4730830
318	dune willow	18592078	4730826
319	dune willow	18592078	4730829
320	dune willow	18592076	4730834
321	dune willow	18592076	4730834
322	dune willow	18592075	4730835
323	dune willow	18592073	4730837
324	dune willow	18592074	4730817
325	dune willow	18592077	4730816
326	dune willow	18592044	4730815
327	dune willow	18592080	4730818
328	dune willow	18592079	4730822
329	dune willow	18592078	4730821
330	dune willow	18592087	4730822

Plant ID Number	Plant Species	UTM (Easterly)	UTM (Northerly)
331	scrub oak	18592079	4730827
332	scrub oak	18592078	4730824
333	scrub oak	18592074	4730820
334	scrub oak	18592069	4730820
335	scrub oak	18592068	4730822
336	scrub oak	18592064	4730821
337	scrub oak	18592066	4730823
338	scrub oak	18592059	4730824
339	scrub oak	18592058	4730823
340	scrub oak	18592058	4730819
341	scrub oak	18592055	4730818
342	scrub oak	18592051	4730819
343	scrub oak	18592053	4730815
344	scrub oak	18592049	4730820
345	scrub oak	18592051	4730824
346	scrub oak	18592045	4730824
347	scrub oak	18592037	4730829
348	scrub oak	18592041	4730828
349	scrub oak	18592044	4730822
350	scrub oak	18592045	4730819
351	scrub oak	18592042	4730814
352	scrub oak	18592041	4730810
353	scrub oak	18592038	4730812
354	scrub oak	18592035	4730811
355	scrub oak	18592030	4730820
356	scrub oak	18592028	4730807
357	scrub oak	18592026	4730811
358	scrub oak	18592031	4730816
359	scrub oak	18592037	4730821
360	scrub oak	18592036	4730823

Plant ID Number	Plant Species	UTM (Easterly)	UTM (Northerly)
361	dwarf chestnut oak	18592016	4730808
362	dwarf chestnut oak	18592019	4730806
363	dwarf chestnut oak	18592018	4730803
364	dwarf chestnut oak	18592015	4730802
365	dwarf chestnut oak	18592008	4730799
366	dwarf chestnut oak	18592004	4730802
367	dwarf chestnut oak	18592005	4730794
368	dwarf chestnut oak	18592005	4730792
369	dwarf chestnut oak	18592004	4730782
370	dwarf chestnut oak	18592013	4730777
371	dwarf chestnut oak	18592006	4730773
372	dwarf chestnut oak	18592008	4730767
373	dwarf chestnut oak	18591998	4730770
374	dwarf chestnut oak	18591992	4730770
375	dwarf chestnut oak	18591995	4730796
376	dwarf chestnut oak	18591991	4730790
377	dwarf chestnut oak	18591985	4730793
378	dwarf chestnut oak	18591988	4730787
379	dwarf chestnut oak	18591972	4730781
380	dwarf chestnut oak	18591981	4730785
381	dwarf chestnut oak	18591976	4730777
382	dwarf chestnut oak	18591974	4730774
383	dwarf chestnut oak	18592016	4730772
384	dwarf chestnut oak	18591976	4730773
385	dwarf chestnut oak	18591977	4730768
386	dwarf chestnut oak	18591980	4730770
387	dwarf chestnut oak	18591984	4730763
388	dwarf chestnut oak	18591987	4730763
389	dwarf chestnut oak	18591996	4730768
390	dwarf chestnut oak	18591992	4730770

APPENDIX B

List of all soil invertebrate taxa identified in control and treatment groups of three shrub species of the Albany Pine Bush Preserve

	Dune Willow *Control*	**Scrub Oak** *Control*	**Dwarf Chestnut Oak** *Control*	**Total Control**	**Dune Willow** *Treatment*	**Scrub Oak** *Treatment*	**Dwarf Chestnut Oak** *Treatment*	**Total Treatment**
Hymenoptera: Formicidae: formica sp.	8	10	15	**33**	2	6	12	**20**
Hymenoptera: Formicidae: Larvae	11	6	0	**17**	4	1	0	**5**
Hymenoptera: other	0	0	1	**1**	0	0	1	**1**
Acari: Ixodidae sp.	29	35	39	**103**	2	20	7	**29**
Acari: Trombiculidae	8	9	17	**34**	0	7	4	**11**
Acari nymph	6	19	42	**67**	0	16	1	**17**
Collembola: Onychiuridae	12	21	14	**47**	0	9	10	**19**
Collembola- Isotimidae sp.	0	0	0	**0**	0	0	3	**3**
Nemotode	2	10	1	**13**	0	2	2	**4**
Arachnidae sp.	1	0	0	**1**	0	0	0	**0**
Thysanoptera	0	1	0	**1**	0	1	15	**16**
Mallophaga	0	1	0	**1**	0	0	0	**0**
Coleoptera	0	4	1	**5**	0	0	2	**2**
Protura	0	1	0	**1**	0	0	0	**0**
Diploda	0	0	1	**1**	0	0	0	**0**
Pseudo-scorpionida	0	0	1	**1**	0	1	1	**2**
Diptera (larvae)	2	1	0	**3**	0	1	0	**1**
Mecoptera (larvae)	1	0	1	**2**	1	0	0	**1**
Orthoptera (larvae)	2	0	0	**2**	0	0	0	**0**
Hemiptera (nymph)	0	1	0	**1**	0	0	0	**0**
Unknown larvae	2	10	3	**15**	2	0	0	**2**
Total	**84**	**129**	**136**	**349**	**11**	**64**	**58**	**133**

APPENDIX C

Summary of growth data for treatment and control groups of dune willow, scrub oak, and dwarf chestnut oak. Assessments were taken one year after treatment. Averages are shown for treatment and control groups of each species, for each group pooled, and for each plant species separately. ANOVA analysis revealed no significant difference between control (N= 225) and treatment (N=225) for either internodal length or diameter.

Group	Plant	Average Diameter	Average Internodal Length
Control	*dune willow*	1.44	23.70
Control	*scrub oak*	3.63	18.46
Control	*dwarf chestnut oak*	2.65	13.96
	Control Average:	**2.57**	**18.71**
Treatment	*dune willow*	1.53	24.01
Treatment	*scrub oak*	3.69	19.12
Treatment	*dwarf chestnut oak*	2.65	15.02
	Treatment Average:	**2.62**	**19.38**